



Attorney Docket No. 24911  
Serial No. 09/509,418

IN THE SPECIFICATION

Please amend the Specification as indicated in the replacement paragraphs and sections below.

On page 5, line 2, please replace the Table with the following:

ANTISENSE OLIGONUCLEOTIDE	SEQUENCE
OLIGO 83 (SEQ ID NO: 1)	<u>TTAAGGATGTTGCCACTGGC</u> <u>GCCAGTGGCAACATCCTTAA</u>
OLIGO 86 (SEQ ID NO: 2)	<u>AATGGCTGTTTACGGCTCTT</u> <u>AAGCACCCCTAACAGGCCATT</u>
OLIGO 90 (SEQ ID NO: 3)	<u>TGTGCCCCCTTGGAGGTGG</u> <u>GCAGCTCCGAGCCGGCCACA</u>
OLIGO 91 (SEQ ID NO: 4)	<u>GGGGGGATGGCTGGGGGGGG</u> <u>GCCGGCCACAGGCATGGCGC</u>
OLIGO 92 (SEQ ID NO: 4)	<u>GGGGGGATGGCTGGGGGGGG</u> <u>GCCGGCCACAGGCATGGCGC</u>
OLIGO 93 (SEQ ID NO: 6)	<u>GGGGGGGGGGGGGGGGGGGG</u> <u>GGCATGGCGCGGGGGGGGG</u>
OLIGO 81 (SEQ ID NO: 10)	<u>CTCACTTGGGGGGGGGGGG</u> <u>CAAATCTGAGGGAGCTGAG</u>
ODN 32 (SEQ ID NO: 7)	<u>ATGGGGCAACGGTTCTTAA</u> <u>TTTAGGAACCGTTGGCGCAT</u>
PAS/TSS (SEQ ID NO: 8)	<u>GGGGGGGGGGGGGGGGGGGG</u> <u>GGCCGGCGCGGCAGCTCCGAGCCGGCCACA</u>
PAS/EXON1,2 (SEQ ID NO. 9)	<u>GGUAGACGGGGGGGGGGGGGG</u> <u>GCAGAGGGATTTCATCTCTCAGGCTGTAGC</u>

On page 11, lines 18-21, please replace the paragraph with the following paragraph:

--Figure 3 shows that HeLa cell growth is inhibited by transfection with antisense TS oligo 83 (targeted to a 3' untranslated sequence downstream of the translation stop site), but is not affected by transfection with antisense TS oligo 81 (targeted to ~~the translation start site~~ a sequence in the 3' untranslated region of TS mRNA). The experimental protocol was as described in the legend to Figure 2.--

On page 12, lines 6-9, please replace the paragraph with the following paragraph:

--Figure 5 shows that transient transfection of HeLa cells with oligo 83 (targeted to a sequence in the 3' untranslated region of TS mRNA) enhances sensitivity to Tomudex whereas oligo 81 (targeted to a 3' sequence downstream of that targeted by oligo 81 83) has no effect on Tomudex sensitivity.--

On page 20, line 6, please replace the line with the following line:

--sense TS (JK-5): CTAGATGTGGCCGGCTCGGAGCTGCCGCGCCGCA (SEQ ID NO: 11)--

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On page 20, line 7, please replace the line with the following line:

--antisense TS (JK-2) : AGCTTGGCCGGCGCGGAGCTCCGAGCCGGCACAT (SEQ ID NO: 12)--

On page 20, line 10, please replace the line with the following line:

--sense TS (JK-3) : CTAGAGCTACAGCCTGAGAGATGAATTCCCTCTGCA (SEQ ID NO: 13)--

On page 20, line 11, please replace the line with the following line:

--antisense TS (JK-4) : AGCTTGCAGAGGAAATTCATCTCTCAGGCTGTAGCT (SEQ ID NO: 14)--

On page 20, line 19, please replace the line with the following line:

--sense MT: CTCTTCAGCACGCCATGGAT (SEQ ID NO: 15)--

Page 20, line 21, please replace the line with the following line:

--antisense MT: AGGGTCTACCTTCTTGCAC (SEQ ID NO: 16)--

Page 25, lines 1-17, please replace the paragraph with the following paragraph:

--hr. The enzyme was inactivated at 95°C for 5 min. The resulting cDNAs (in a volume of 2.5  $\mu$ l) were amplified in a polymerase chain reaction (PCR) using 1.25 U of *Taq* DNA polymerase in 50  $\mu$ l of 20 mM Tris-HCl pH 8.4), 50 mM KCl, 0.1 nM mixed dNTPs, 2 mM MgCl<sub>2</sub>, and 50 pmol of primers specific for TS and GAPDH cDNAs. TS and GAPDH cDNAs were amplified together in the same reaction tube to allow the level of housekeeping GAPDH cDNA to be used to determine the relative level of TS mRNA. Twenty-four to 27 cycles of PCR amplification (94°C 45 s, 55°C 30 s, 72°C 90 s) produced fragments of 208 bp and 752 bp using primer sets for TS (forward 5' CACACTTGGGAGATGCACA3' (SEQ ID NO: 17); reverse 5' CTTGAAAGCACCTAAACAOCCAT3' (SEQ ID NO: 18)) and GAPDH (forward 5' TATTGGGCGCTGGTCACCA3' (SEQ ID NO: 19); reverse 5' CCACCTTCTTGATGTCATCA3' (SEQ ID NO: 20), respectively. PCR products were separated on a 1.2% agarose gel, and transferred to Hybond nylon membrane (Amersham, Canada, Ltd., Oakville, Ontario, Canada) by Southern blotting. Blots were hybridized to [ $\alpha$ -<sup>32</sup>P]dCTP random primer-labeled probe (pcHTS-1, a generous gift from Dr. K. Takeishi, University of Shizuoka, Shizuoka, Japan; or a cDNA insert recognizing glyceraldehydes-3-phosphate dehydrogenase [GAPDH]).

Hybridisation signals were quantified using a PhosphorImager and ImageQuant (Molecular Dynamics, Sunnyvale, California, USA).--

Page 25, lines 20-31, please replace the paragraph with the following paragraph:

--Cellular content of TS was assayed by binding of [6-<sup>3</sup>H]5-FdUMP. This method was demonstrated to label total TS unless the cells were pretreated with 5-FU or 5-FudR. The assay was performed using cells that were treated with antisense ODN 83 or the scrambled control ODN ~~82~~ 32. Briefly, cells were harvested by scraping into PBS and resuspending the subsequent pellet in 100 mM KH<sub>2</sub>PO<sub>4</sub> (pH 7.4). Cells were disrupted by freezing and thawing, followed by sonication. The total protein concentration was determined using Coomassie staining (BioRad reagent) (MI) in order to express results as pmol 5-FdUMP bound per mg total protein. 5-FdUMP binding was assessed in paired lysates from cells transfected with ODN 83 or ODN 32, in separate incubation reactions carried out on different days; however, pairs were always assessed together under the same reaction conditions. On each occasion, the incubation vessel contained 50 µg of total protein, 75 µM methylene-FH<sub>4</sub>, 100 mM mercaptoethanol, 50 mM KH<sub>2</sub>PO<sub>4</sub> (pH 7.4), and 15 nM [6-<sup>3</sup>H]5-FdUMP in a final--